Effect of aqueous extract of *Ipomoea carnea* leaf on isolated frog and mouse heart

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*Ipomoea carnea* fam. Convolvulaceae is a poisonous plant and its toxicity is supposed to be due to the cardiac and respiratory failure. The present paper describes the cardiac effect of aqueous extract of the fresh leaves of *I. carnea* using mouse and frog heart. The aqueous extract produced an initial blockade of isolated frog heart for 5-10 sec followed by dose dependent increase in both amplitude and rate that lasts up to 2 min. Atropine (1 μg/ml) blocked the initial depressant phase and potentiated the stimulant effect of the aqueous extract. The dose dependent increase in cardiac contractility of aqueous extract was not altered by propranolol or calcium channel blockers like nifedipine or diltiazem. The decrease in sodium chloride concentration or increase in potassium chloride concentration in physiological salt solution inhibited the responses to aqueous extract while an increase in sodium chloride concentration or decrease in potassium chloride or calcium chloride concentration in physiological salt solution potentiated the responses to the aqueous extract of *I. carnea*. It may be suggested from the data that aqueous extract of *I. carnea* produces positive inotropic effect on isolated frog heart possibly by sodium extrusion or release of the intracellular calcium.

*Ipomoea carnea* Var. Jacq. family Convolvulaceae (Hindi-Behaya) is a long struggling shrub growing all over India. It is toxic to the live stock and toxicity is supposed to be due to cardiac and respiratory failure. A water-soluble carbohydrate, ipomose isolated from the plant shows positive test for aldoses and ketoses and seems to be a new tri and tetra saccharide. Leaves contain anthracene glycoside and sapotoxins. *I. carnea* leaf extract showed no significant autonomic, CNS and behavioral effects in experimental mice; except sedation, decrease motor activity, distressed respiratory failure. It is reported that *I. carnea* is devoid of anticonvulsant, analgesic and antipyretic activity against mites (*Psorotic* spp) and the lice (*Liognathus* spp) of buffaloes. *I. carnea* decreases the values of cholinesterase but does not affect blood cholesterol, blood urea nitrogen, blood sugar, sodium, potassium and calcium levels, total serum protein, (albumin, globulin and albumin-globulin ratio). The whole plant’s aqueous extract is also reported to be useful in rheumatic diseases. In the present investigation we have investigated the effects of aqueous extract of *I. carnea* leaves on isolated frog and mouse heart.

**Materials and Methods**

Fresh leaves of *Ipomoea carnea* were collected in month of May from Sangvi, Pune and authenticated from Botanical Survey of India, Pune. The washed leaves were dried in shade at room temperature (25° to 30°C). The dried leaves were powdered in hammer mill and passed through sieve no. 80. The powder was moistened with the demineralised water (1500 ml). The extraction process was carried out for the period of 24 hr. The extract was filtered and concentrated slowly at 30° to 40°C over hot plate, in preweighed glass beaker. The process of heating and cooling of residue was continued till a constant weight of mass was obtained. The residue was dissolved in distilled water and diluted as per requirement.

Frogs (*Rana Tigerina*) of either sex weighing 100-150 g were pithed to spinal cord, to the level of third vertebra. The heart was quickly exposed and inferior vena cava was cleaned and ‘V’ shape cut was made on inferior vena cava near heart and Syme’s cannula was inserted into it. The heart along with Syme’s cannula was isolated from the body and fixed on a stand. The Syme’s cannula was connected to the reservoir containing frog Ringer solution (pH 7.4) and continuously bubbled with air. The flow rate of frog Ringer solution was kept at 4-5 ml/min by means of screw clip for about 15 min prior to administration of any dose. The responses to aqueous extract were recorded on smoke drums using Sterling’s heart lever, that was connected to ventricle of heart and adjusted to give ten times’ magnification.

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In first series of experiments, after 15 min of stabilization, graded doses of aqueous extract (600 to 4800 µg/ml) of *I. carnea* leaves were added and responses were recorded. The high dose was added only after the recovery from the preceding dose. Usually responses recovered within 5 min. To study the interaction of aqueous extract with other agents, the heart was perfused with the solution containing β-adrenergic blocker propranolol (2 µg/ml), calcium channel blockers like the diltiazem (1 µg/ml), nifedipine (0.3 µg/ml), verapamil (0.3 µg/ml) and cholinergic blocker atropine (1 µg/ml) for 15 min and responses to aqueous extract were recorded as mentioned before.

In second series of experiments, the perfusion fluid was replaced by frog Ringer solution containing half sodium chloride (55 mM) or double sodium chloride (220 mM), half potassium chloride (0.9 mM) or double potassium chloride (3.6 mM) or half calcium chloride (0.79 mM) or double calcium chloride (3.17 mM) concentration. To maintain the isomolarity, either sucrose was added or glucose concentration was reduced wherever necessary to half. The heart perfused for 15 min with these solutions and the responses to extract were re-elicited as usual.

In another series of experiment, ECG recordings of mouse heart, treated with the various doses of aqueous extract were carried out. For this purpose, albino mice obtained from Hindustan Antibiotics Limited, Pimpri of either sex were selected for the experiment. Mice were anaesthetized with freshly prepared thiopental sodium solution (35 mg/kg, iv.). When animals under full anaesthesia were stabilized (which took about ten min), they were placed on wooden board, on its back and special electrocardiographic electrodes for small animals were kept in intimate contact with the flexor aspect of limb and secured in position with help of adhesive strips. The electrodes in turn were connected to junction box and it was connected to the Student’s physiograph with common cord to EKG cauvet. The speed of physiograph was kept at 50 mm per second. Initially the sensitivity of instrument was standardized and initial control readings were taken for all leads, viz. I, II, III, aVR, aVL, and aVF. The aqueous extract was administered (40 and 160 mg/kg, iv.) and readings were taken immediately after administration and thereafter at the end of 15, 30, 45, and 60 min. At the end of the experiment the electrodes were detached from the limbs of animals and animals were returned to the respective cages and nursed till recovery.

**Results**

The aqueous extract produced initial blockade of isolated frog heart for 5-10 sec followed by increase in both rate and amplitude that lasted for about 2 min. At high doses (above 8 mg) the aqueous extract showed arrest of the heart beats. The responses to the aqueous extract were not affected by propranolol (2 µg/ml), diltiazem (1 µg/ml) and nifedipine (0.3 µg/ml). The stimulant responses of the aqueous extract were blocked by verapamil (0.3 µg/ml) (Fig. 1A). The initial depressant phase induced by the aqueous extract of *Ipomoea carnea* was blocked by the atropine (1 µg/ml) (Fig. 1B). It has also
potentiated the stimulant responses of the aqueous extract.

When the concentration of the sodium chloride was decreased (from 110 to 55 mM) or potassium chloride (from 1.8 to 3.6 mM) or calcium chloride concentration was increased (from 1.58 to 3.16 mM), the responses to aqueous extract were significantly reduced. It was also noted that the increase in concentration of sodium chloride (from 1.58 to 0.79 mM) caused a significant reduction in the responses to aqueous extract (Fig. 2).

The results of EEG recordings (Table 1) of mouse heart showed biphasic responses at dose (40 mg/kg, iv) showed positive chronotropic effect while at dose (160 mg/kg, iv) showed negative inotropic effect.

**Discussion**

The results of the present studies reveal that the aqueous extract produces initial blockade of isolated frog heart for 5-10 sec followed by an increase in both amplitude and rate which lasted for about 2 min at doses (600-4800 µg) and at high doses (above 8 mg) produced complete heart blockade.

The mechanism involved in the positive inotropic action of the various drugs, may be the direct stimulation of adrenergic receptors as seen with catecholamines or indirect sympathomimetic action involving release of noradrenaline as seen with dopamine, fatty acids, diuretics, insulin etc. or through direct action on the muscle.

Aqueous extract induced positive inotropic effects were neither altered by propranolol, the β-adrenergic receptor antagonist, nor by calcium channel blockers like diltiazem and nifedipine.

In the present study, three different calcium channel blockers were used because of their different mechanism of actions. The site of action of calcium channel blockers may be the sarcolemma membrane or intracellular structure. Verapamil prevented norepinephrine induced contraction in calcium free medium, suggestive of a possible effect on intracellular calcium transport while diltiazem and nifedipine acts on sarcolemmal structure. The responses to aqueous extract were significantly inhibited when calcium concentration was increased to double and response to extract was potentiated.

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**Table 1—Effect of aqueous extract of Ipomoea carnea (40 mg and 160 mg/kg, ip) on electrocardiograph recordings of mouse heart**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Immediately after administration</th>
<th>15 min. after administration</th>
<th>30 min. after administration</th>
<th>45 min. after administration</th>
<th>60 min. after administration</th>
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<tbody>
<tr>
<td>Heart Rate</td>
<td></td>
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<tr>
<td>40 mg/kg</td>
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<tr>
<td>PR</td>
<td>0.04 ± 0.004</td>
<td>0.037 ± 0.005</td>
<td>0.043 ± 0.002</td>
<td>0.034 ± 0.009</td>
<td>0.033 ± 0.011</td>
<td>0.034 ± 0.013</td>
</tr>
<tr>
<td>QRS Interval</td>
<td>0.053 ± 0.002</td>
<td>0.051 ± 0.008</td>
<td>0.049 ± 0.010</td>
<td>0.041 ± 0.023</td>
<td>0.044 ± 0.015</td>
<td>0.041 ± 0.008</td>
</tr>
<tr>
<td>QT Interval</td>
<td>0.025 ± 0.01</td>
<td>0.02 ± 0</td>
<td>0.021 ± 0.002</td>
<td>0.024 ± 0.008</td>
<td>0.021 ± 0.002</td>
<td>0.023 ± 0.0046</td>
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<tr>
<td>PT Interval</td>
<td>0.088 ± 0.011</td>
<td>0.083 ± 0.005</td>
<td>0.081 ± 0.010</td>
<td>0.073 ± 0.012</td>
<td>0.072 ± 0.029</td>
<td>0.068 ± 0.024</td>
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<tr>
<td>PR</td>
<td>0.04 ± 0</td>
<td>0.041 ± 0.0023</td>
<td>0.039 ± 0.0105</td>
<td>0.039 ± 0.0023</td>
<td>0.033 ± 0.0023</td>
<td>0.029 ± 0.009</td>
</tr>
<tr>
<td>QRS Interval</td>
<td>0.04 ± 0</td>
<td>0.048 ± 0.0105</td>
<td>0.041 ± 0.0105</td>
<td>0.033 ± 0.014</td>
<td>0.033 ± 0.012</td>
<td>0.033 ± 0.012</td>
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<tr>
<td>QT Interval</td>
<td>0.116 ± 0.143</td>
<td>0.024 ± 0.017</td>
<td>0.033 ± 0.014</td>
<td>0.033 ± 0.012</td>
<td>0.029 ± 0.016</td>
<td>0.028 ± 0.011</td>
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<tr>
<td>PT Interval</td>
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<td>0.1 ± 0.02</td>
<td>0.09 ± 0.008</td>
<td>0.09 ± 0.030</td>
<td>0.09 ± 0.030</td>
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</table>
when calcium ion concentration was reduced to half. This effect can not be due to change in osmolarity as the equimolar concentration of sucrose was added. Thus it is reasonable to assume that stimulant response of aqueous extract, may be due to its action on the intracellular structure that regulates calcium release.

The initial depressant phase induced by aqueous extract was blocked by the atropine and later in the stimulant phase, it has potentiated the inotropic effect of the extract. This indicated that the initial depressant phase was due to the cholinergic receptor activation. The responses to aqueous extract were significantly inhibited when sodium ion concentration was reduced to one half. This could not due to change in osmolarity because of addition of equimolar concentration of sucrose in place of sodium. Thus indicates that aqueous extracts produces inotropic effect in isolated frog heart by facilitating sodium influx. On the other hand, the responses to aqueous extract were significantly inhibited when potassium ion concentration was increased to double and vice versa. This indicates that the extracellular concentration of KCl activates the Na⁺-K⁺ ATPase. All the negative results have been mentioned in the text, but its extensive data has not been given here to avoid the unnecessary details.

In conclusion, it is suggestive from data that aqueous extract induced positive inotropic effects are probably due to sodium extrusion mechanism or release of Ca²⁺ from intracellular structure. The initial depressant phase seems to be due to the activation of the cholinergic receptors.

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References